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# *TP53* mutation profile of esophageal squamous cell carcinomas of patients from Southeastern Brazil

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ABSTRACT

Esophageal cancer (EC) is among the 10 most common and fatal malignacies in the world, presenting a marked geographic variation in incidence rates between and within different countries. The *TP53* tumor suppressor gene is highly mutated in esophageal tumors and its mutation pattern can offer clues to the etiopathology of the tumor. As Brazil presents one of the highest incidence areas in the West, a deeper knowledge of the molecular mechanisms related to EC development in the Brazilian population is needed. We analyzed the mutation profile of 110 esophageal squamous cell carcinomas (ESCC) of patients from Southeastern Brazil (Rio de Janeiro and São Paulo) and collected data regarding alcohol intake and tobacco smoking. We detected 41 mutations in tumor samples from 38 patients. There was no association between mutation frequency and tobacco smoking or alcohol drinking. The most frequently mutated codons were 179, 214, 220 and 248. Codons 179, 220 and 248 are hot-spots for ESCC, but codon 214 presents only 0.7% of the mutations registered in the IARC database. The mutation profile revealed a high percentage of mutations at A:T base pairs (34.1%) followed by deletions (17.1%). We concluded that the mutation profile detected in this study is different from that of patients from Southern Brazil but very similar to that previously seen in French patients, being characterized by a high frequency of mutations at A:T base pairs, which may be associated with acetaldehyde, the metabolic product of ethanol.


## 1. Introduction

Esophageal cancer is the eighth most common and the sixth most fatal cancer in the world. Prognosis for this disease is poor, with a general 5-year survival rate around 10–15%, mainly due to the late stages at which it is diagnosed [1,2]. Although a recent increase in the incidence rate of esophageal adenocarcinoma has been observed, most esophageal tumors are squamous cell carcinomas (ESCC) [3].

There is a marked geographical variation in ESCC incidence, with high incidence areas in the East, in the so-called Asian cancer belt, stretching from Iran to China, and in certain places in the West, such as France and Brazil. The differences in incidence rates suggest a pivotal role of environmental carcinogens in tumor development [1,2].

Different etiologic factors have been associated with ESCC in various regions. Alcohol consumption and tobacco smoking are the major risk factors for ESCC in Western countries [1,2,4]. Consumption of hot beverages, with the consequent thermal irritation and inflammation, among other factors, has been associated with the disease in the East and also in defined regions of Western countries [4,5].

In Brazil, ESCC is the fourth most fatal malignancy among men and the sixth among women, being responsible for over 8000 deaths each year [6]. The highest incidence rates are found in the Southern and Southeastern regions of the country [6]. Although tobacco and alcohol consumption are associated with the disease throughout the country [6–8], the consumption of a hot beverage, hot-maté, is a risk factor only in Southern Brazil, since it is a local habit [9].

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The development of ESCC involves multiple genetic alterations, the most common being mutations in the *TP53* gene [10,11]. The *TP53* gene is a tumor suppressor gene located on chromosome 17p13 and encodes a nuclear 53 kDa phosphoprotein (p53) [12]. This protein is involved in DNA repair, cell cycle control, angiogenesis, and apoptosis [13,14]. More than 70% of the alterations reported to date occur in the DNA-binding domain of p53, which is encoded by exons 5–8 of the *TP53* gene [15]. Most of these alterations are missense point mutations [16] and, frequently, the mutant protein has no transactivational activity due to an impaired DNA-binding activity [17,18]. Mutations in the *TP53* gene are considered an early event in ESCC development and the mutation profile differs according to different geographical areas, providing clues to the mechanisms involved in mutagenesis and carcinogenesis [12].

There is no data in the literature regarding *TP53* alterations in ESCC samples obtained from patients living in Southeastern Brazil. Hitherto, the only ESCC *TP53* mutation profile described in Brazil was from patients residing in Southern Brazil [19]. Therefore, the aim of this study was to characterize *TP53* mutations in tumor samples from Brazilian patients living in Rio de Janeiro and São Paulo, and to compare this mutation profile with that from Southern Brazil. We show that the mutation profile in our ESCC samples is more similar to that seen in France than in Southern Brazil.

## 2. Materials and methods

### 2.1. Patients

One-hundred and ten patients with a histologically confirmed diagnosis of ESCC were recruited between September/2000 and August/2008 from 3 hospitals situated in the Southeastern region of Brazil: Hospital Universitário Pedro Ernesto (HUPE-UERJ) and Instituto Nacional de Câncer (INCA), both located at Rio de Janeiro, and Hospital de Clínicas-Gastrocentro (HC-UNICAMP), located at Campinas, São Paulo. All individuals who took part in this study signed an informed consent and information was obtained by a standardized questionnaire, including data on tobacco smoking and alcohol drinking. Data on tobacco smoking was obtained concerning the number of cigarettes smoked and the duration of the habit (expressed as pack/year, defined as the number of packs smoked per day multiplied by the number of smoking years), and individuals were classified as never or ever-smokers (defined as smoking at least one cigarette per day and persisting for more than 1 year). Individuals were classified regarding alcohol intake as never or ever-drinkers (defined as drinking alcoholic beverages at least twice a week and persisting for more than 1 year), and data was also collected about the type of alcoholic beverage regularly consumed. Data related to tumor differentiation and esophageal localization were also collected. Eighteen surgical and 92 endoscopic biopsy samples were stored at −20°C until use. Samples were also collected from the adjacent non-tumoral esophageal mucosa. All analyses were done both in the tumor and in the normal mucosa. The study proposal and all ethical proceedings were approved by the Ethic Committees of the 3 hospitals.

### 2.2. DNA isolation, TP53 exon amplification and direct sequencing

DNA was extracted from samples by proteinase K/SDS digestion as described elsewhere [20,21]. Amplification of *TP53* (exons 5–8 and exon–intron boundaries) was done as previously described [22], with some modifications. After amplification, PCR products were analyzed by direct DNA sequencing. Before sequencing, PCR products were purified using the GFX PCR DNA and Gel Band Purification Kit (GeHealthcare Life Sciences, São Paulo, SP, Brazil). To minimize sequencing artifacts induced by PCR, products of at least two different PCR amplification reactions were sequenced using forward and reverse primers with the DYEnamic ET Dye Terminator Cycle Sequencing Kit and analyzed on a MegaBace 1000 automated sequencer (GeHealthcare Life Sciences, São Paulo, SP, Brazil). All the reactions (forward and reverse) were done as quadruplicates. To determine whether the mutations occurred in homo- or heterozygosis each chromatogram was carefully inspected. When superimposed peaks were detected in at least three separate chromatograms in each direction, the mutations were marked as heterozygous. When mutations occurred as a single peak in at least three separate chromatograms in each direction, they were marked as homozygous. All chromatograms were compared to a *TP53* reference sequence (genebank accession X54156). The description of the base and codon numbers of exons 5–8 is as follows:

Exon 5–184 bp, 62 codons (from 126 to 187), from genomic base 13,055 to 13,238.
Exon 6–113 bp, 37 codons (from 187 to 224), from genomic base 13,320 to 13,432.
Exon 7–111 bp, 36 codons (from 225 to 261), from genomic base 13,993 to 14,109.
Exon 8–137 bp, 45 codons (from 261 to 307), from genomic base 14,452 to 14,588.

### 2.3. Statistical analysis

All statistical analyses were performed using the software GraphPad Instat (GraphPad Software, Inc., San Diego, CA, USA). Differences were considered statistically significant when $P < 0.05$, using Fisher's exact test.

The prevalence and the pattern of *TP53* mutations obtained for patients from Southeastern Brazil were compared with compiled data reported for esophageal squamous cell carcinoma in the IARC *TP53* mutation database, November 2008 release, R13 [16]. We included only the results found between exons 5 and 8, excluding polymorphisms and other intronic regions that are not the splicing sites.

## 3. Results

In this study, esophageal samples from primary ESCC were examined for *TP53* mutations in 110 patients. Gender, tobacco smoking, alcohol drinking, and tumor differentiation were documented for 110 (100%), 108 (98.2%), 108 (98.2%), and 100 (90.9%) patients, respectively. Table 1 shows that most patients enrolled in this study were males (83.6%), and the mean ages were $59.0 \pm 10.0$ (range 33–82) and $61.4 \pm 9.4$ (range 43–75) for males and females, respectively. Most patients were regular alcohol drinkers (ever-drinkers: 91.7%) and smokers (ever-smokers: 90.7%), and the vast majority (94.9%) consumed both tobacco and alcohol. Most patients (80.0%) consumed alcohol as cachaça, a sugar cane spirit, and smoked industrialized tobacco (90.5%).

PCR amplification and direct sequencing revealed 41 mutations in tumor samples from 38 patients (Table 2). The prevalence of *TP53* mutations was, therefore, 34.5% (38/110 tumors). No mutations were found in normal tissues. Four patients (45, 178, 190 and 194) were polymorphic at codon 213 (CGA to CGG; data not shown) and no other polymorphism was found. There were no correlations between the frequency of *TP53* gene mutations and the variables of gender, age, alcohol drinking, or tobacco smoking. Furthermore, there was no difference in cigarette consumption between patients who presented tumors with or without *TP53* mutations. There was no association between the frequency of *TP53* mutations and the type of alcoholic drink consumed.

Most *TP53* alterations were found in exon 5 (34.1%), followed by exon 7 (24.3%), exon 6 (22%), and exon 8 (12.2%). Three mutations (7.3%) occurred at splicing sites. Among these, there were 33 (80.5%) point mutations, including 25 missense (61%) and 5 nonsense mutations (12.2%), 7 deletions (17.1%) and one 15 bp insertion (2.4%). Curiously, there was a high frequency of deletions (17.1%) detected, with three of them being single base and four being 10–18 bp deletions, mostly occurring between codons 155–180 and 250–275. The most frequently mutated codons were 248, with three mutations in two different bases (two were 14070G to A and one was 14069C to T), 179 (both mutations at 13215A, with one to G and the other to T), 214 (both mutations at 13402T to G, with one tumor having a further mutation 13401A to G), and 220 (both mutations were 13374A to G).

The pattern of *TP53* alterations revealed a high frequency of mutations at A:T base pairs (both transitions and transversions, 34.1%), followed by deletions (17.1%) (Table 3). The G:C to A:T and G:C to T:A mutations represented, each, 14.6% of the total number of mutations, with G:C to A:T at CpG representing 12.2%. There was no significant difference in the mutation pattern frequency of light and non-smokers (we joined these two groups, since there was a low number of patients in each of these categories) when compared with heavy-smokers. However, the number of transitions was almost two-fold more frequent in light and non-smokers as compared to heavy-smokers (5/8, 63%, in non-and light-smokers; and 10/27 in heavy-smokers, $P = 0.246$).

Fig. 1 shows the comparison of the mutation profile of ESCC patients enrolled in this study with those from Southern Brazil [19] and also from France and China (all registered in the IARC *TP53* database) [16]. The results show that Brazilian Southeast-

**Table 1**
Clinicopathological characteristics of 110 patients with ESCC (%)[a].

| | Total | *TP53* mutated | *TP53* not mutated | *P*-value[b] |
|---|---|---|---|---|
| Number | 110 | 38 | 72 | – |
| Gender | | | | |
| Male | 92 (83.6%) | 32 (84.2%) | 60 (83.3%) | – |
| Female | 18 (16.4%) | 6 (15.8%) | 12 (16.7%) | 1.00 |
| Age | | | | |
| Male | 59.0 ± 10.0 | 56.4 ± 10.7 | 60.4 ± 9.4 | – |
| Range | (33–82) | (33–76) | (39–82) | 0.085 |
| Female | 61.4 ± 9.4 | 62.5 ± 4.2 | 60.8 ± 11.3 | – |
| Range | (43–75) | (56–67) | (43–75) | 0.651 |
| Tobacco smoking | | | | |
| Never | 10 (9.3%) | 3 (8.1%) | 7 (9.9%) | – |
| Ever | 98 (90.7%) | 34 (91.9%) | 64 (90.1%) | 1.00 |
| Pack/year | | | | |
| <20 | 10 (11.8%) | 5 (15.6%) | 5 (9.4%) | – |
| ≥20 | 75 (88.2%) | 27 (84.4%) | 48 (90.6%) | 0.492 |
| Mean ± S.D. | 50.5 ± 30.8 | 55.5 ± 35.8 | 47.5 ± 27.3 | 0.282 |
| Alcohol drinking | | | | |
| Never | 9 (8.3%) | 3 (8.1%) | 6 (8.5%) | – |
| Ever | 99 (91.7%) | 34 (91.9%) | 65 (91.5%) | 1.00 |
| Alcohol drinking/tobacco smoking | | | | |
| Never/Never | 5 (5.1%) | 3 (8.1%) | 2 (3.3%) | – |
| Ever/Ever | 93 (94.9%) | 34 (91.9%) | 59 (96.7%) | 0.363 |
| Tumor differentiation | | | | |
| Well | 7 (7.0%) | 1 (2.9%) | 6 (9.1%) | – |
| Moderate | 65 (65.0%) | 25 (73.5%) | 40 (60.6%) | 0.410 |
| Poor | 22 (22.0%) | 7 (20.6%) | 15 (22.7%) | 0.635 |
| Undifferentiated | 6 (6.0%) | 1 (2.9%) | 5 (7.6%) | 1.00 |

[a] Total may vary due to missing data.
[b] *TP53* mutated compared with not mutated.

ern patients presented the highest frequency of deletions (17.1%) (Fig. 1A). The high frequency of A:T base pair (A:T to C:G, A:T to G:C, and A:T to T:A) point mutations detected in this study (34.1%) was similar to that of French patients (38.1%) ($P = 0.693$), and considerably higher than those from Southern Brazil (16.7%, $P = 0.084$) and China (13.8%, $P = 0.002$). The frequency of G:C to A:T (both, at CpG and non-CpG) point mutations was also very similar between Southeastern Brazilian (26.8%) and French patients (26.3%) ($P = 1.00$), and lower (particularly those at non-CpG) than those of Southern Brazilian (41.7%) ($P = 0.182$) or Chinese (41.4%) ($P = 0.091$) patients. When we joined the mutations at A:T, G:C at CpG, and G:C non-CpG base pairs, it is clearly observed that the mutation profile of Southeastern Brazilian patients is much more similar to French than to Southern Brazilian patients (Fig. 1B).

## 4. Discussion

In this study we analyzed *TP53* mutation patterns in ESCC of 110 patients living in Southeastern Brazil (Rio de Janeiro and São Paulo). The prevalence of mutations was 34.5%, which is similar to that found in Southern Brazil [19], but lower than what has been seen in France (over 80%) [23,24] or in Eastern countries, such as China (42–70%) [25–28] and Iran (50–65%) [29,30].

There was no association between *TP53* mutation prevalence and cigarette consumption, even though other studies have revealed that ESCC from smokers show a higher prevalence of mutations than non-smokers, and that this frequency was directly associated with the number of cigarettes consumed [11,31–33]. A possible reason for this might be the low number of non-smokers (less than 10%) and light-smokers (9%) enrolled in this study.

The main codons mutated in this study were 179, 214, 220, and 248. Codons 179, 220 and 248 are hot-spots for ESCC, but codon 214 presents only 8 out of 1173 (0.7%) mutations registered in the IARC database, with 3 of these being in French patients (3/71, about 4%) [16]. The profile of these two mutations is also different from those registered in the IARC database, with one being in tandem (CAT to CGG) and the other being a transversion (CAT to CAG), whereas those registered in the IARC database were one deletion, one transversion (CAT to GAT) and six transitions (CAT to CGT). We did not detect mutations at only two (175 and 282) of the other 6 hot-spots for ESCC (codons 175, 176, 193, 245, 273, and 282). Interestingly, we detected a mutation in codon 193 and another in codon 194. Codons 193, 194, and 195 are rarely mutated in tumors in general, but make up almost 4% of mutations in ESCC in general and 6% in ESCC from French patients. There were two codons mutated in this study that had never been detected before: codon 147 (GTT to GAT) and codon 229 (TGT to TGA).

Codons 245, 248, and 273 are highly mutated in lung tumors from smokers, presenting mainly G to T mutations [33]. However, as previously noticed for other tobacco-related tumors, the mutations we detected in these codons were not G to T, but instead G:C to A:T. Furthermore, we did not detect point mutations at codons 157 and 158, the other 2 hot-spots in lung tumors from smokers [33], suggesting that the mechanisms of tobacco-related *TP53* mutations in lung and esophagus may be different. In this regard, in a previous study [34] which analyzed the expression of cytochromes P450 (CYP) in human esophagus, we did not detect the expression of CYP1A1, the enzyme expressed in lung [35] and responsible for activating benzo[a]pyrene, suspected to originate the G to T transversions in lung tumors from smokers [33].

The mutation profile detected in this study showed a high percentage of mutations at A:T base pairs, which can be associated with acetaldehyde, the metabolic product of ethanol [36]. This agrees with the fact that over 90% of the patients that took part in this study consumed alcohol, particularly as cachaça, a traditional Brazilian

**Table 2**
*TP53* mutations in exons 5–8 in ESCC from Southeastern Brazilian patients.

| DNA number | Age | Gender | Skin color | Alcohol drinking | Tobacco smoking | Pack/year | Exon number | Mutation[a] | Codon number | Genomic position | Amino acid change | Nature of mutation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 67 | Male | Non-white | Ever | Ever | 3 | 7 | del −10 | 252–255 | 14,081–14,090 | Leu → NA | Deletion |
| 22 | 33 | Male | White | Ever | Ever | 17 | 6 | TAT to TGT | 205 | 13,374 | Tyr → Cys | Missense |
| 24 | 52 | Male | Non-white | Ever | Ever | 80 | 8 | del −1 | 272 | 14,483 | Val → NA | Deletion |
| | | | | | | | 8 | GCC to GAC | 276 | 14,496 | Ala → Asp | Missense |
| 37 | 65 | Female | White | Never | Never | 0 | 7 | AGG to AGT | 249 | 14,074 | Arg → Ser | Missense |
| 40 | 68 | Male | White | Ever | Ever | 72 | 5 | del −1 | 158 | 13,151 | Arg → NA | Deletion |
| 45 | 68 | Male | White | Ever | Ever | 46 | 7 | CGG to TGG (CpG) | 248 | 14,069 | Arg → Trp | Missense |
| | | | | | | | 5 | GGC to GTC | 154 | 13,140 | Gly → Val | Missense |
| 64 | 56 | Male | White | Ever | Ever | 100 | 5 | CCC to TCC | 151 | 13,130 | Pro → Ser | Missense |
| 65 | 44 | Male | White | Ever | Ever | 48 | 5 | del −14 | 157–161 | 13,148–13,161 | Val → NA | Deletion |
| 67 | 53 | Male | White | Ever | Ever | 80 | 8 | del −1 | 262 | 14,455 | Gly → NA | Deletion |
| 81 | 60 | Male | White | Never | Never | 0 | 5 | CAT to CGT | 179 | 13,215 | His → Arg | Missense |
| 83 | 68 | Male | Non-white | Ever | Ever | 96 | 5 | CAT to CTT | 179 | 13,215 | His → Leu | Missense |
| 85 | 61 | Female | ND | Ever | Ever | 100 | 5 | GTT to GAT | 147 | 13,119 | Val → Asp | Missense |
| 137 | 51 | Male | Non-white | Ever | Ever | 74 | 6 | TAT to TGT | 220 | 13,374 | Tyr → Cys | Missense |
| 138 | 47 | Male | White | Ever | Ever | 62 | Intron 5 | G to A | – | 13,319 | – | Splice |
| 139 | 54 | Male | Non-white | Ever | Ever | 10 | 7 | TGT to TGA | 229 | 14,014 | Cys → Stop | Nonsense |
| 140 | 56 | Female | Non-white | Ever | Ever | 23 | 7 | dupl 15 bp | 250–254 | 14,075–14,089 | Ins Ile, Leu, Ter, Ile, Ile | other |
| 141 | 65 | Male | White | Ever | Ever | 39 | 5 | ATG to AAG | 133 | 13,077 | Met → Lys | Missense |
| 146 | 69 | Male | Non-white | Ever | Ever | 28 | 6 | CAT to CGG | 214 | 13,401–13,402 | His → Arg | Missense |
| 148 | 54 | Male | Non-white | Ever | Ever | 154 | Intron 6 | G to T | – | 13,433 | – | Splice |
| 150 | 74 | Male | Non-white | Ever | Ever | 14 | 6 | CAT to CGT | 193 | 13,338 | His → Arg | Missense |
| 152 | 55 | Male | Non-white | Ever | Ever | 40 | 5 | GTT to TTT | 172 | 13,193 | Val → Phe | Missense |
| 153 | 41 | Male | White | Ever | Ever | 30 | 7 | ATC to TTC | 255 | 14,090 | Ile → Phe | Missense |
| 154 | 67 | Female | Non-white | Ever | Ever | 51 | 5 | CAG to TAG | 167 | 13,178 | Gln → Stop | Nonsense |
| 156 | 56 | Male | Non-white | Ever | Ever | 39 | 6 | CTT to CCT | 194 | 13,341 | Leu → Pro | Missense |
| 163 | 66 | Female | Non-white | Never | Never | 0 | 7 | GGC to AGC (CpG) | 245 | 14,060 | Gly → Ser | Missense |
| 165 | 41 | Male | Non-white | Ever | Ever | 4 | 8 | CGT to TGT (CpG) | 273 | 14,486 | Arg → Cys | Missense |
| 168 | 48 | Male | Non-white | Ever | Ever | 38 | 7 | CGG to CAG (CpG) | 248 | 14,070 | Arg → Gln | Missense |
| 169 | 56 | Male | ND | ND | ND | ND | 6 | CAG to TAG | 192 | 13,334 | Gln → Stop | Nonsense |
| 174 | 62 | Male | Non-white | Ever | Ever | OT | 7 | ACA to CCA | 256 | 14,093 | Thr → Pro | Missense |
| 175 | 76 | Male | Non-white | Ever | Ever | 56 | 8 | GAA to CAA | 286 | 14,525 | Gly → Gln | Missense |
| 178 | 51 | Male | White | Ever | Ever | 52 | 6 | TAT to TGT | 220 | 13,374 | Tyr → Cys | Missense |
| 180 | 54 | Male | Non-white | Ever | Ever | 60 | 5 | del −18 | 174–180 | 13,200–13,217 | Arg → NA | Deletion |
| 181 | 60 | Female | Non-white | Ever | Ever | 50 | 5 | TGC to TTC | 176 | 13,206 | Cys → Phe | Missense |
| 183 | 74 | Male | Non-white | Ever | Ever | 134 | 5 | del −10 | 162–165 | 13,164–13,173 | Ile → NA | Deletion |
| 209 | ? | Male | Non-white | Ever | Ever | 92 | 5 | CAG to TAG | 165 | 13,172 | Gln → Stop | Nonsense |
| | | | | | | | Intron 5 | G to A | – | 13,239 | – | Splice |
| 211 | 44 | Male | Non-white | Ever | Ever | 29 | 7 | CGG to CAG (CpG) | 248 | 14,070 | Arg → Gln | Missense |
| 216 | 56 | Male | White | Ever | Ever | OT | 6 | AGA to TGA | 209 | 13,385 | Arg → Stop | Nonsense |
| 217 | 50 | Male | White | Ever | Ever | 55,5 | 6 | CAT to CAG | 214 | 13,402 | His → Gln | Missense |

ND: not determined, missing data; NA: not applicable; OT (other type): refers to pipe to patient 174 and to hand-made cigarettes to patient 216.
[a] Mutation is detailed as the codon base change or as del (deletion) and the number of bases deleted, or as dupl (duplication) and the number of base pairs duplicated.

**Table 3**
*TP53* mutation pattern in ESCC samples from Southeastern Brazil (%).

| Mutation type | Southeastern Brazil (RJ and SP) |
|---|---|
| A:T to C:G | 2 (4.9%) |
| A:T to G:C | 6 (14.6%) |
| A:T to T:A | 6 (14.6%) |
| G:C > A:T | 6 (14.6%) |
| G:C > A:T at CpG | 5 (12.2%) |
| G:C > C:G | 1 (2.4%) |
| G:C > T:A | 6 (14.6%) |
| Deletions (1 bp) | 3 (7.3%) |
| Deletions (>1 bp) | 4 (9.8%) |
| Deletions (all) | 7 (17.1%) |
| Other[1] | 2 (4.9%) |

[1] *Other*: Includes the tandem mutation of patient 146 and the 15 bp duplication of patient 140.

sugar cane spirit that has a high ethanol concentration (around 45%).

We also found a high frequency of deletions (17.1%). Interestingly, they occur in two clusters, from codon 157 to 180 (57.1%) and from codon 250 to 272 (42.9%), regions showing a predominance of G:C base pairs lying in the vicinity of CpG sites. By comparison, 18.7% and 8.7% of the deletions registered in ESCC tumors in the IARC database are at the former and latter regions, respectively [16].

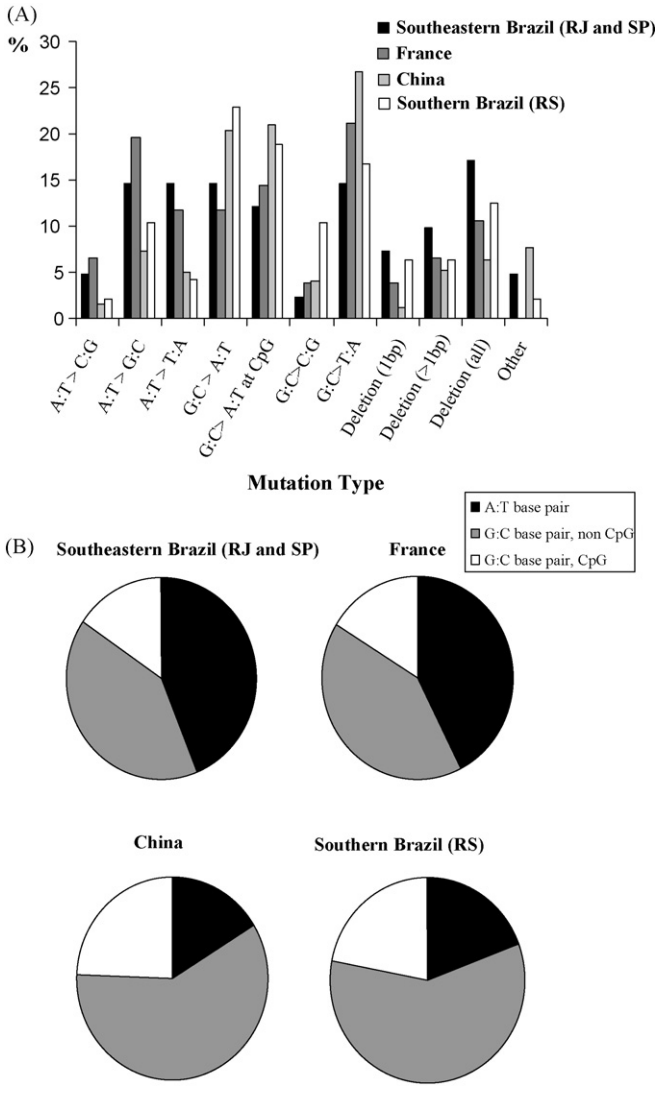


**Fig. 1.** Comparison of the *TP53* mutational spectrum between ESCC of patients from Southeastern Brazil, the IARC *TP53* database (France and China) and Southern Brazil (RS), either by specific mutation profile (A) or by base pair mutation profile (B).

The *TP53* mutation profile of ESCC from Southeastern Brazilian patients was highly similar to that of French [16], and clearly different from that observed in patients from Southern Brazil [19] and China [16]. ESCC from French and Southeastern Brazilian patients presented a high percentage of A:T base pair mutations. Instead, Southern Brazilian and Chinese patients presented a high percentage of mutations at G:C base pairs. This may be due to the different etiological factors associated with the disease in these areas. Alcohol and tobacco consumption are the etiological factors associated with ESCC in France [4,37] and Southeastern Brazil [6–8]. Although these two factors have also been associated with ESCC in Southern Brazil [38], the consumption of hot-maté, a locally common habit, has also been strongly linked with the disease in this part of Brazil [9]. In China, the consumption of hot beverages is also a risk factor for ESCC [39]. Another risk factor historically associated with ESCC in China is the presence of nitrosamines in food, particularly in high incidence areas [40]. The high percentage of G:C to A:T mutations present in ESCC from Southern Brazilian patients was suggested by Putz et al. [19] to be caused by nitrosamines, although the source of exposure was not identified. The percentage of G:A to A:T mutations at CpG was highly similar among the 4 areas, since it is not likely to be related to any particular exposure, but rather to endogenous background events.

Nevertheless, this study suggests that the mutation profile of ESCC from Southeastern Brazilian patients is different from that of Southern Brazil but very similar to that previously seen in France, being characterized by a high frequency of mutations at A:T base pair, which may be associated with acetaldehyde, the metabolic product of ethanol.

## Conflict of interest

The authors of the present study hereby certify that they do not possess any conflict of interest that could inappropriately influence the work presented.

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